# The Need for Speed - Rapid Methodologies to Determine Bathing Beach Water Quality

## The Problem

*The Jones family of Bayside, California, goes to the beach on **Saturday**. But the Bayside Health Department won't know until **Sunday** if the water is safe for swimming!*

*Enterococci* colonies on mEI agar

## Why?

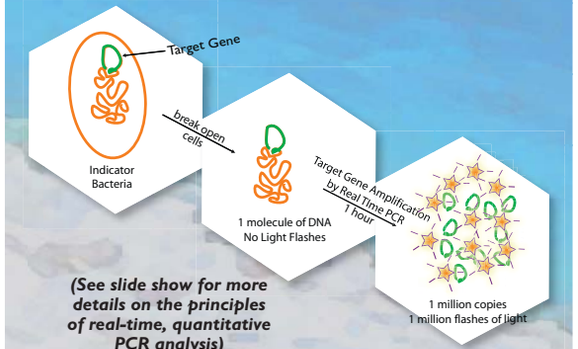
*Current microbiological methods designed to determine if it is safe to swim at marine and freshwater beaches require 24 hours for results to become available.*

## The Solution

*The U.S. EPA National Exposure Research Laboratory in Cincinnati, OH and U.S. EPA New England Regional Laboratory in Chelmsford, MA are developing/evaluating rapid, and real-time PCR (Polymerase Chain Reaction) methods for the quantitative detection of fecal bacteria in recreational waters*. These methods can give results in as little as 2 hours so that people will know before they go whether it is safe to swim.*

**The presence of fecal bacteria in recreational waters indicates that pathogenic bacteria, viruses and/or protozoan parasites might also be present.*

# Real Time PCR




*(See slide show for more details on the principles of real-time, quantitative PCR analysis)*

*MagNA Pure*

*The U.S. EPA National Exposure Research Laboratory in Cincinnati is conducting tests using the Smart Cycler® system from Cepheid, Inc. This instrument is portable, easy to use and can perform analyses in as little as ~20 minutes. Sample preparation is being performed by a simple, manual method (see computer slide show) that can be completed in a matter of minutes.*

*The U.S. EPA New England Regional Laboratory in Massachusetts is conducting testing using the Light Cycler® system from Roche Applied Science. This instrument can also generate results in as little as ~20 minutes. Sample preparation is being performed using a MagNA Pure robotic instrument from Roche Applied Science that allows hands-off, high sample throughput in about 1 hour.*

*Light Cycler®*

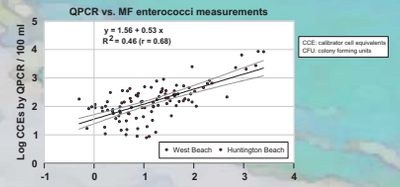
# ORD - National Exposure Research Laboratory studies in 2003

*Last summer the USEPA Office of Research and Development launched a new 3-year epidemiology study to evaluate the correlations between various adverse health outcomes reported by swimmers and beach water quality as determined by three new methodologies.*

*These methodologies included an improved membrane filtration (MF) culture method and a rapid QPCR method for detecting Enterococcus fecal indicator bacteria.*

*The first year study sites included West Beach at the Indiana Dunes National Lakeshore in Porter, Indiana on Lake Michigan and Huntingon Beach in Bay Village, Ohio on Lake Erie.*






*The overall correlation coefficient between the results from the MF and QPCR methods was 0.68. Given the strong correlation that has been previously demonstrated between swimming-related illness rates and Enterococcus MF results, this correlation between the results of the two methods offers promise that the QPCR method may also be a useful tool for determining health risks due to fecal contamination at freshwater recreational beaches.*

*Direct analyses of the correlation between the QPCR analysis method results and swimmer illness rates in the 2003 epidemiology study are currently in progress.*

# U.S. EPA, Region 1 studies in 2003

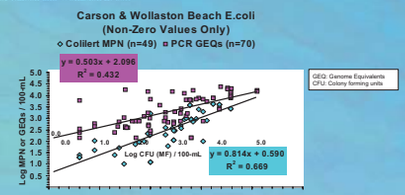
*Last summer the New England Regional Laboratory initiated field testing of a high throughput DNA isolation and real-time PCR protocol for Escherichia coli and Enterococcus fecal indicator bacteria in the first phase of a bacterial source tracking study in marine waters.*

*The primary testing objective was to quantify and correlate the levels of viable indicator bacteria and their genomic DNA in these waters.*

*Water sampling sites were located at Carson Beach in Boston, Massachusetts on North Dorchester Bay and at Wollaston Beach in Quincy, Massachusetts.*



Regression analysis graph of Carson and Wollaston *E. coli*




*The overall correlation coefficient between the results from the MF and QPCR methods was 0.66. This correlation between the results of the two methods suggests that the QPCR method will also be a useful tool for the quantitative analysis of fecal indicator bacteria in marine waters.*